



Example

Cultivation of *Streptomyces clavuligerus*

Vegetative phase in pre-seed tank

Media for pre-seed tank

Volume of pre-seed tank = 500 l

Volume of media = 350 l

Composition	amounts
corn starch	7.0 kg
soybean flour	7.0 kg
NaH ₂ PO ₄	0.185 kg
Estol (Priolube 1435)	0.35 kg
synperonic	0.35 kg
tap water	to 350 l

It was processed in an analogous manner as in example 1

Vegetative phase in seed fermenter

Media for seed fermenter

Volume of seed fermenter = 7500 l

Volume of media = 4500 l

Composition	amount
corn starch	90.0 kg
soybean flour	90.0 kg
NaH ₂ PO ₄	2.4 kg
Estol (Prolube 1435)	4.5 kg
synperonic	4.5 kg
tap water	to 4500 l

It was processed in an analogous manner as in example 1

Fed batch fermentation of *Streptomyces clavuligerus* ATCC 27064

Media for fermenters

Volume of fermenter = 90 000 l

Volume of media = 65 000 l

Composition	amount
corn starch	1300 kg
soybean flour	2600 kg
NaCl	130 kg
Estol (Priolube 1435) *	1500 kg
NaH ₂ PO ₄	5.4 kg (0.0083%)
MgCl ₂ . 6 H ₂ O	7.8 kg
FeCl ₃ . 6H ₂ O	3.5 kg
ZnCl ₂	0.45 kg
CuCl ₂ . H ₂ O	0.2 kg
MnSO ₄ . H ₂ O	0.3 kg
synperonic	65 kg
tap water	to 65 m ³

Legend:

- Estol is a generic name for glycerol trioleate; (Priolube is registered trade mark owned by company Unichem GmbH, Germany)
- Synperonic (registered trade mark owned by ICI, GB) antifoam agent base on propylenglycol
- * soybean oil can be used instead of Estol

The content of a culture of *Streptomyces clavuligerus* ATCC 27064 in the vegetative phase of growth from the seed fermenter were used to inoculate by a sterile transfer into a sterile starting medium (65 000 l) of above composition in a 90 000 l stainless steel fermenter equipped for mixing and a delivery of sterile air through filters with a 0.2 µm pore size. The fermentation media and all inlet-pipes were sterilized and cooled by sterile air to 24°C. The fermentation phase from seed fermenter was maintained at 24°C to 25°C and 0.3 Bar. The broth was mixed at 62 rpm and carried out under aeration conditions that provide for a dissolved oxygen concentration between 30% to 40% during the course of whole fermentation and pH of the media was maintained by addition of aqueous solution of sodium hydroxyde (NaOH) at value 6.8 to 6.9. The fermentation lasted for 148 hours and the resultant concentration of clavulanic acid was 4410 µg/ml.

During the course of the fermentation of *Streptomyces clavuligerus* ATCC 27064 a source of phosphorus - sodium dihydrogen phosphate (NaH₂PO₄) were added up to 40 hours in the growth phase. An aqueous solution of 80 kg NaH₂PO₄ in 900 l water

was prepared and added during growth phase to the fermentation medium according to the following flow rates:

time (h)	flow rate
5-9	13
10-14	22
15-19	35
20-24	45
25-29	54
30-40	10

The following addition may be added:

glycerol: constant flow rate of 50 l/h (33% aqueous solution) from 30 hours to 148 hours

estol : flow rate of 10 l/h at controlling the concentration between 2-3 g/l to 5 g/l.

Concentration of phosphorus source (NaH_2PO_4) in the fermentation broth during the growth phase up to 40 hours, nitrogen conc. and concentration of clavulanic acid during the fermentation

time (hours)	Source of phosphorus (NaH_2PO_4) concentration		nitrogen conc. g/l	Concentration of clavulanic acid in the fermentation broth $\mu\text{g/ml}$
	(mg/l)	(%)		
0	30	0.003	0.036	
10	30	0.003	0.044	
16	20	0.002		
22	6	0.0006	0.004	
28	4.5	0.00045		310
34	4.5	0.00045	0.12	540
40	3	0.0003		810
43				
46			0.1	1160
49				
52				1400
58			0.15	1810
64				2200
67				
70			0.21	2610
76				2830
82			0.21	3140
94			0.35	3460
100				3590
106			0.41	3700
118			0.42	3980
127				4100
136				4130
142			0.38	4370
148				4410